Summary of the Project "Field Investigations of Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) at the Hanford 100-H Area" conducted by LBNL, PNNL, and Regenesis, Ltd.

Based on the discussions during the LBNL-PNNL-Regenesis videoconference held on March 1, 2005

Funding Sources:

- August 2002—September 2004: DOE Natural and Accelerated Bioremediation (NABIR) Program and EM Office of Science and Technology, and
- Since October 2004: NABIR Program (3 year project).

Project website: http://www-esd.lbl.gov/ERT/hanford100h/index.html

1. Overall Objective and Hypothesis

Overall Objective: To carry out field investigations to assess the potential for immobilizing and detoxifying chromium-contaminated groundwater using lactate-stimulated bioreduction of Cr(VI) to Cr(III) at the Hanford Site's 100-H Area field site.

Hypothesis: Lactate (Hydrogen Release Compound—HRC^{TM}) injection into chromium contaminated groundwater through an injection well will cause indirect or direct bioreduction of chromate [Cr(VI)] and precipitation of insoluble species of [Cr(III)] on soil particles, probably catalyzed at oxide surfaces, at the field scale.

2. Types of Investigations Performed

We have conducted a series of bench-scale and field-scale integrated treatability studies, including the following types of investigations:

1. Microcosm bench-scale study of Hanford sediments, using different types of HRC products and Metal Remediation Compound (MRC).

2. Two new wells—injection Well 699-96-45, located 15 ft downgradient from the existing monitoring well 699-96-43, and a monitoring and pumping Well 699-96-44, located 15 ft downgradient from the injection well, were drilled and equipped at the Hanford 100-H Area field site.

3. Each borehole was completed using a newly developed assembly, including expandable (with argon gas) rubber packers, groundwater samplers, and an inner geophysical access tube.

2. Three Br-tracer injection tests and two pumping tests (concurrently with the Br-tracer tests) were performed before the HRC injection to assess the background hydraulic properties of the Hanford formation and to design the HRC injection test.

3. Pre-HRC injection and post-HRC injection geophysical (seismic and radar) cross-borehole measurements were performed.

4. Pilot field-scale biostimulation of the groundwater was conducted by injecting 40 lbs of ^{13}C-labeled HRC into the injection Well 699-96-45 (over the depth interval from 44 ft to 50 ft within the Hanford formation) on August 3, 2004. Immediately following the HRC injection, we started pumping from the monitoring Well 699-96-44 (which continued until August 30) and injected a Br-tracer solution into the injection well.

5. Pre- and post-HRC injection groundwater sampling was performed from 4 out of 5 groundwater samplers (the uppermost sampler was located above the water level in 2003) in each borehole. During pumping, samples of pumped groundwater were collected and on-site measurements using the Hydrolab [dissolved oxygen (DO), pH, redox potential, electrical conductivity, and temperature] were performed. Hydrolab measurements were also conducted after pumping ceased.

6. Groundwater sampling was conducted initially weekly and then weekly to monthly following termination of pumping.

7. Microbial analyses of groundwater samples included: Acridine orange direct counts and molecular analyses—PLFA, 16S GeneChip, and Clone library, and qPCR.

8. Analytical analyses of groundwater samples included bromide (tracer added to the injection well), chloride and phosphate (added to HRC), acetate (byproduct of HRC microbial metabolism, nitrate and sulfate (present in groundwater under background conditions.

9. Analytical analyses of metals in filtered groundwater samples included Cr(VI), total Cr, and Fe(II) and total Fe.

10. Groundwater samples were analyzed for carbon, nitrate, and oxygen isotopic compositions.

3. Main Results

We have investigated coupled hydraulic, geochemical, and microbial conditions, which are necessary to maximize the extent of Cr(VI) bioreduction and minimize the Cr(III) reoxidation in groundwater.

1. Using bench-scale studies, we have shown that in the Hanford Site's 100-H Area site sediments:

- Several types of bacteria are present in the sediments, including *Arthrobacter, Oxalobacter, Sporomusa* and *Pseudomonas* species, which are known to reduce or absorb hexavalent chromium or other metals

- Under background conditions, the total microbial population is $<10^5$ cells g^{-1}, which is likely insufficient for significant direct enzymatic Cr(VI) reduction.

- Different types of hydrogen release compound (HRC^{TM}) and metal remediation compound (MRC^{TM}) could generate biostimulation and an increase in biomass to $>10^8$ cells g^{-1}, generate highly reducing conditions, and enhance Cr(VI) removal from the pore solution.

2. A discriminate analysis was used to analyze the crosshole geophysical data and the results of borehole flowmeter measurements to estimate a hydraulic conductivity zonation within the Hanford formation.

3. Pilot field-scale biostimulation of the groundwater shows microbial cell counts reached the maximum of 2×10^7 cells g^{-1} 13 to 17 days after the injection. The HRC injection generated highly reducing conditions: DO dropped from 8.2 to 0.35 mg/l, redox potential from 240 to -130 mV, and pH from 8.9 to 6.5.

4. After pumping stopped (under conditions of natural regional groundwater flow):

- DO, redox, and pH began to recover to background values.
- High biomass in groundwater lasted for 2 months and then decreased to values even less than those under pre-HRC-injection conditions. PLFA and direct counts both indicated similar biomass changes; however, the PLFA also indicated an increase during the last 2 months at one depth in Well 699-96-45.
- Carbon isotope ratios of DIC decreased, but remained above background in Well 699-96-44 and within the injection interval in Well 699-96-45 until December 2004.

5. No measurable methane was detected in samples tested. No methanogens were detected by 16S rDNA or by PLFA.

6. PLFA indicated low microbial diversity under background conditions, which increased after injection and continued to increase for the first 6 weeks, followed by the decrease in the microbial diversity. A similar pattern was observed using the 16S rDNA chip analyses.

7. The isotopic composition of nitrate is consistent with that of natural background sources (not agricultural origin) with minor modification due to biodegradation. Low oxygen isotope ratios may indicate high concentrations of nitrite.

8. Geophysical investigations show that HRC products (such as lactic acids) injected into groundwater can be detected using radar and seismic survey, and that even small variations in hydrogeological heterogeneity may influence the distribution of the amendment and its products. A reaction halo of elevated electrical resistivity persisted in front of the injection zone during pumping, and diminished after cessation of pumping. Although our investigations are still underway, our working hypothesis is that this halo indicates a zone of precipitation of metals, such as iron and chromium.

9. $\delta^{13}C$ ratios in dissolved inorganic carbon confirmed microbial metabolism of HRC. $\delta^{13}C$ ratios remain elevated (above background values) after 6 months. Increases in carbon isotope ratios of DIC in Well 44 are coincident with increases in bromide, chloride and acetate and decreases in nitrate. The source of chloride was determined to be the HRC.

10. Hydrogen sulfide production was first observed after about 20 days post-injection, which corresponds with the enrichment of a *Desulfovibrio* species (sulfate reducer) identified using 16S rDNA microarray and monitored by direct fluorescent antibodies. DO and nitrate began to return to background concentrations two months after HRC injection, despite planktonic densities remaining high ($>10^7$ cells/ml).

11. Cr(VI) concentrations in the monitoring and pumping wells decreased significantly and remained below up-gradient concentrations even after 6 months, when redox conditions and microbial densities had returned to background levels.

4. Needs and Directions of Ongoing and Future Research.

First Priority Needs

- Develop a master table of all types of work conducted since the beginning of the project.
- Additional analyses of chromium concentration in groundwater samples, especially under background conditions. (Using PNNL duplicate samples, and additional analyses from LBNL archived samples)
- Stable isotope analysis of Cr to assess the role of microbial versus chemical reduction in groundwater (LBNL).
- Conduct a new pumping test with concurrent sample and data collection to assess whether some HRC remained in the area between the injection and monitoring wells.
- Explore the possibility of using the CPT for drilling and sediment sample collection.

- Drill 3 to 4 new wells to: (1) collect sediment samples to confirm the distribution of the HRC plume between the injection and observation wells, and (2) determine the natural local groundwater flow direction.
- Update the project web page.

Directions of Ongoing and Future Research

- Determine whether dissolved oxygen and manganese oxides (that are present in sediment minerals) could reoxidize Cr(III) to Cr(VI), including the following tasks:
  - Conduct pumping from Well 699-96-44 to assess the longevity of HRC injected in groundwater in 2004; and
  - Use the stable isotopic analysis and Cr-isotopic analysis of water samples to assess the potential for Cr reoxidation.

- Develop a 3D reactive transport code, TOUGHREACT-BIO, to simulate coupled biological and geochemical processes.

5. Conclusions

Microbial, geophysical, and geochemical analyses of groundwater coupled with stable isotope monitoring allowed for accurate tracking of microbial processes during this field treatability study, and confirmed that Cr(VI) was successfully removed from groundwater at a contaminated site using HRC as an electron donor and a carbon source.